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Stereoselective Syntheses of 3′-Hydroxyamino- and 3′-Methoxyamino-2′,3′-Dideoxynucleosides

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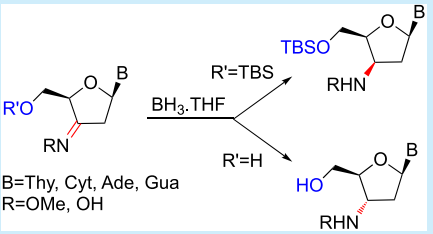
Supporting Information

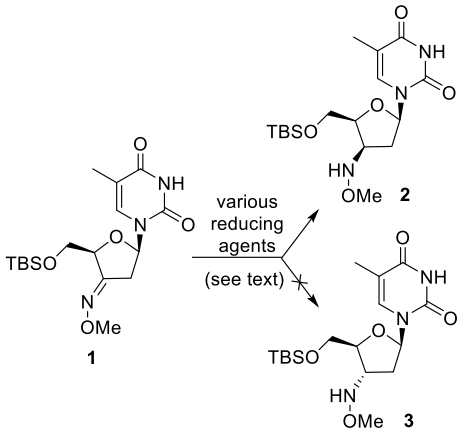
ABSTRACT: Aminonucleosides are used as key motifs in medicinal and bioconjugate chemistry; however, existing strategies toward 3′-hypernucleophilic amine systems do not readily deliver *deoxyribo*-configured products. We report diastereoselective syntheses of *deoxyribo*- and *deoxyxylo*-configured 3′-hydroxyamino- and 3′-methoxyamino-nucelosides from 3′-imine intermediates. The presence or absence of the 5′-hydroxyl-group protection dictates facial selectivity via inter- or intramolecular delivery of hydride from BH_3 (borane). Protecting group screening gave one access to previously unknown 3′-methoxyamino-deoxyguanosine derivatives.



Amino-functionalized nucleosides are key fragments for the development of antiviral agents, nucleic acids technologies, and bioconjugates. While the introduction of *aza*-functionalities at the 5′-position is relatively straightforward because of the limited effect of steric hindrance, 3′-functionalization is more challenging. Modified *ribo*- and *deoxyribo*-nucleosides with hydroxyamino and methoxyamino groups at their 3′-positions possess antiviral, anti-leukemic, and anti-HIV activities.[1] For example, the growth of L1210 cells was shown to be inhibited by 2′-deoxy-2′-(hydroxyamino) cytidine with an IC_{50} of 1.84 μM; however, synthesis was achieved indirectly, via a uridine derivative.[1b] Tronchet et al.[2] explored the synthesis of 3′-methoxyamino- and 3′-hydroxyamino-derivatives by stereoselective reduction of 3′-imines. They readily obtained *deoxyxylo*-configured systems as major or exclusive products across a range of reduction conditions. The *deoxyribo*-isomers, on the other hand, were usually minor products or absent, where syntheses have only been achieved via indirect, multistep methods. Richert, Szostak, and their co-workers have also exploited the nucleophilicity of amines for chemical primer extension studies; however, they have not taken advantage of the enhanced nucleophilicities of hypernucleophilic amines.[3] Thus, we sought to develop a stereoselective reduction strategy to access *deoxyribo*-configured 3′-hydroxyamino- and 3′-methoxyamino-nucleoside systems directly from 3′-imine intermediates.

Our initial investigations centered on thymidine systems because they do not require nucleobase protection and show reasonable solubility properties. We chose 5′-*O*-TBDMS-2,3-dideoxy-3-*N*-methoxyimino-thymidine **1** as our starting material, and it was prepared according to reported procedures.[4,2a] Tronchet et al.[2a] reported the use of $NaBH_3CN$ to reduce **1**, albeit with low levels of conversion; thus, we explored the use of $Bu_3SnH/BF_3{\cdot}Et_2O$,[5] L-selectride,[6] and $NaBH_4$;[7] however, in all cases, we were unable to obtain the desired *ribo*-configured compound **3** (Scheme 1), and the *xylo*-product was formed instead.

Scheme 1. Several Hydride-Transfer Agents Were Explored and Each Delivered Deoxyxylo-Configured Product 2 Exclusively



Sebesta et al.[8] and Matsuda and co-workers[1b] successfully synthesized 2′-(alkoxyamino)uridines via the intramolecular nucleophilic substitution upon 2,2′-*O*-anhydrouridine derivatives. Thus, we attempted nucleophilic substitution at the 3′-position of 2,3′-anhydrothymidine with methoxylamine under a range of reaction conditions; however, surprisingly, we only observed a hydrolytic opening of the anhydro-linkage.

Stereoselective reduction of 3′-keto nucleosides to ribonucleosides via intramolecular delivery of hydride, tethered through a free 5′-hydroxyl group, has been reported.[9] Moreover, Matsuda and co-workers[1b] reported that 3′-(hydroxyamino) uridine with a *ribo*-configuration **5a** can be obtained from the corresponding 3′-hydroxyiminouridine **4a** by treatment with $NaBH_4$/AcOH (Scheme 2). Thus, we

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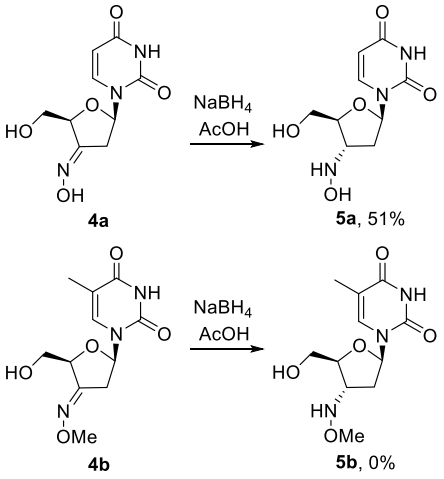
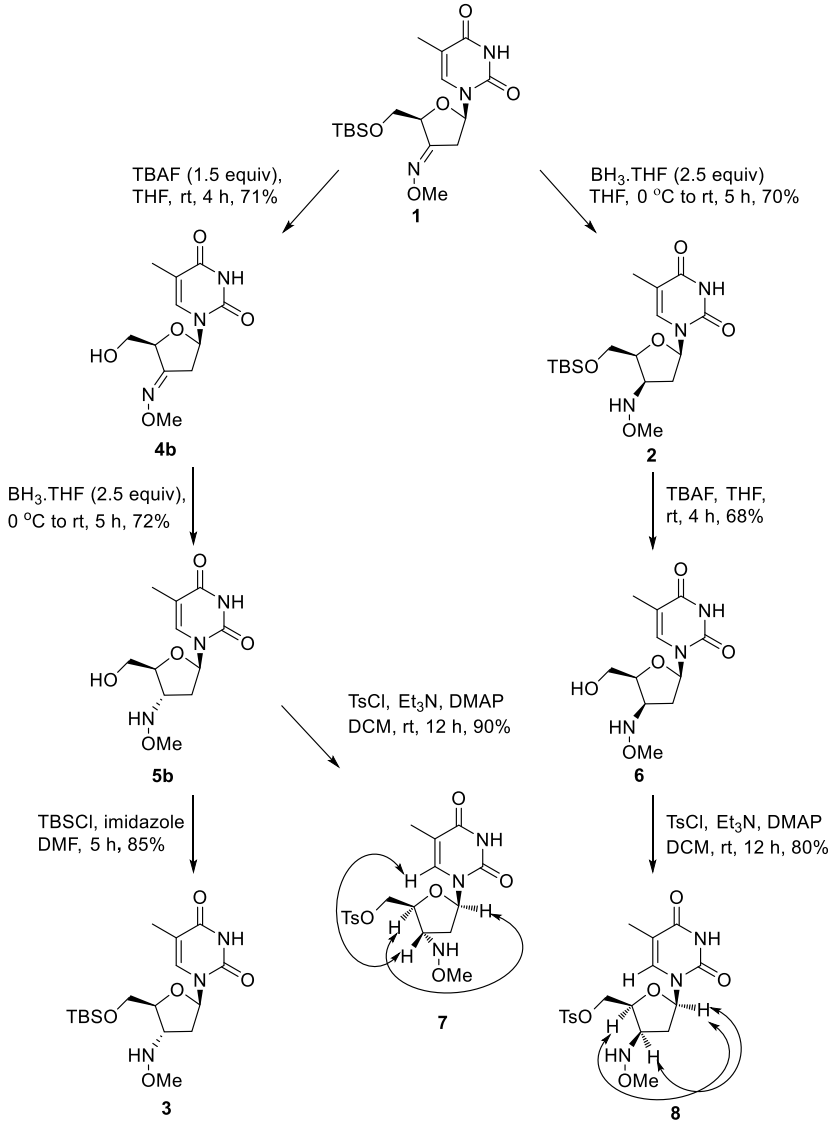
Scheme 2. Stereoselective Reduction of Uridine-Based Oxime 4a[1b] Is Observed but Not for the Thymidine Analog 4b



attempted the reduction of imine **4b** under similar conditions; however, poor conversion to **5b** was observed (Scheme 2). This result aligns with the findings of Tronchet et al.,[2] who used $NaBH_3CN$ upon **1** under acidic conditions to obtain low levels of the deoxyribomethoxyamino-product **5b** as part of a complex mixture that prevented the isolation of pure material.

We then explored the application of the borane–tetrahydrofuran complex for the reduction of **4b**, which we expected to show higher reactivity and higher levels of conversion. To our delight, we obtained 3′-methoxyamino-thymidine **5b** with the desired *deoxyribo*-configuration exclusively in 72% yield (Scheme 3). We were also able to reduce protected imine **1** with BH_3·THF to give *deoxyxylo*-configured product **2** in a yield of 70%. We sought to confirm the absolute configurations of the deprotected 3′-methoxyamino-products **5b** and **6** by 2D NMR spectroscopy. Unfortunately, the signals arising from the 3′-H [NCH(OMe)], 4′-H (OCH), and the 5′-H (OCH_2OTBS) protons were overlapping in the ^1H NMR spectra, thus preventing clear assignments by NOESY correlations. We also attempted

Scheme 3. Stereoselective Syntheses of *Deoxyribo*- and *Deoxyxylo*-Configured 3′-Methoxyamino-Thymidines[a]



[a]Arrows on structures **7** and **8** indicate observed NOESY correlations.

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similar analyses using the 5′-TBS-protected systems **2** and **3**; however, we encountered the same signal overlap problems. Thus, in order to increase the chemical shifts of the 5′-H signals and, to a lesser extent, 4′-H signals, we prepared 5′-tosyl derivatives **7** and **8**. This strategy allowed us to distinguish and assign each of the proton signals around the sugar rings. The *deoxyribo*-isomer **7** did not show NOESY correlation between the 3′- and the 1′-protons, whereas correlations were clearly observed for the *deoxyxylo*-isomer **8**. Additionally, in the case of *deoxyribo*-isomer **7**, NOESY signals were observed between the 3′-proton and thymine nucleobase, along with the expected NOESY correlation between the 4′- and the 1′-protons. The *xylo*-isomer **8** also showed the expected 4′−1′ NOESY correlations.

In order to gain mechanistic insights into the proposed intramolecular hydride delivery via complexation of the boron to the free hydroxyl group at the 5′-position, we carried out ^{11}B NMR experiments.[10] The 5′-TBS protected thymidine imine **1** and deprotected 3′-methoxyimino thymidine **4b** were treated with $B(OMe)_3$ in THF-d_8. Starting with the addition of 0.5 equiv of $B(OMe)_3$, ^{11}B NMR spectra were recorded for multiple additions of 0.5 equiv of $B(OMe)_3$ up to 2.5 equiv. Figure 1 gives evidence for B−N complexation via the imine

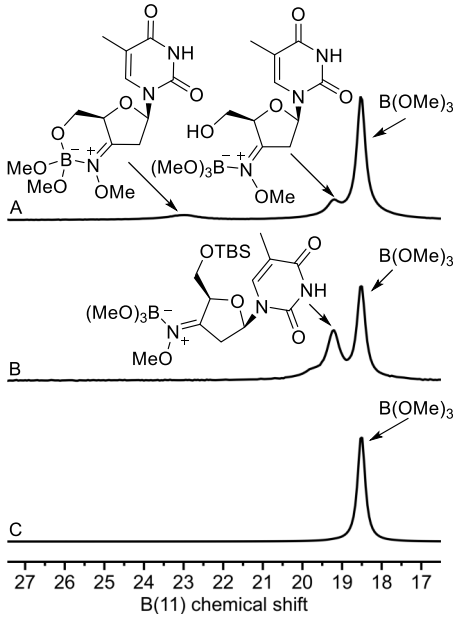

Figure 1. ^{11}B NMR studies in THF-d_8. (A) 5′-OH imine **4b** (1.0 equiv) mixed with $B(OMe)_3$ (1.5 equiv). (B) 5′-OTBS imine **1** (1.0 equiv) mixed with $B(OMe)_3$ (1.5 equiv). (C) $B(OMe)_3$ alone.

nitrogen of 5′-TBS-protected 3′-methoxyimino-thymidine **1** via a signal at 19.19 ppm, which persists even after overnight incubation with 2.5 equiv of $B(OMe)_3$. In the case of the 5′-hydroxy 3′-methoxyimino-thymidine **4b**, we observed two distinct signals at 22.98 ppm (RO−B−N) and 19.20 ppm that indicate the complexation of boron with the free hydroxyl group at the 5′-position and B−N complex, respectively (Figure 1).[11] Taken together, these simple experiments support the idea of a critical role for 5′-OH complexation in the reduction of **4b** to deliver the *deoxyribo*-configuration observed in **5b**.

On the basis of our promising results with the thymidine system, we applied the same strategies to the adenosine and cytidine systems. Reduction with BH_3·THF was successfully performed on 5′-OH- and 5′-OTBS-3′-methoxyimino-2′,3′-dideoxycytidine systems[12] to afford *deoxyribo*-product (**9a**) and *deoxyxylo*-product (**9b**), respectively, in 71% and 68% yields (Figure 2). The 5′-OH-3′-methoxyimino-2′,3′-dideox-

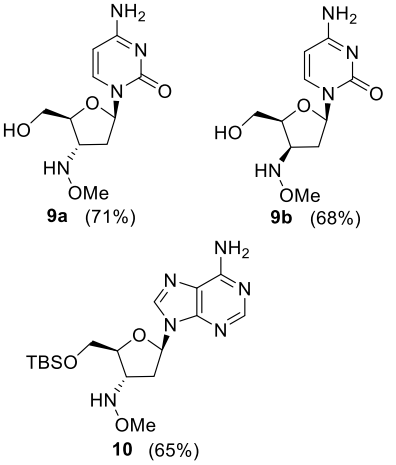

Figure 2. Product scope for deoxycytidine and deoxyadenosine systems.

yadenosine system[12] afforded the deoxyribomethoxylamine product **10** exclusively, which was derivatized at the 5′-position (Figure 2) to minimize conformational changes and, thus, confirm configuration (see the Supporting Information).[14,2b]

We then moved on to explore the application of our BH_3·THF reduction strategies toward guanosine systems. Guanosine systems present significant synthetic challenges because of their poor solubility properties.[13] With this in mind, we attempted reductions on the 5′-OTBS-*N*-isobutyroyl-protected methoxyimino-derivative of deoxyguanosine and the analogous 5′-OH system[12] using BH_3·THF. These reactions resulted in the reduction of the imines to the desired *deoxyxylo*-product (**11b**) and *deoxyribo*-product (**11a**) in 85% and 70% yield, respectively, but the isobutyroyl group was also reduced. Thus, we moved to a *N*-DMT-protected substrate, which tolerated BH_3·THF to yield the *deoxyribo*-product **12** after TBS protection, as its tosic acid salt in 80% yield upon deprotection of the DMT group (Figure 3). The configurations of the derivatives of all guanosine products were confirmed by NOESY analysis of the 5′-derivatives (see the Supporting Information).

Next, we explored the BH_3·THF reductions of 3′-hydroxyimino systems. The unprotected 3′-hydroxyimino-thymidine derivative[4] **13a** was reduced by BH_3·THF stereoselectively to give *deoxyribo*-configured **14a**[15] as the major product alongside the *deoxyxylo*-derivative **14b**[1c] in a 4:1 ratio, where the mixture could be separated by column chromatography. On the other hand, the 5′-TBS-protected 3′-hydroxyimino-thymidine derivative **13b**[2b] afforded the *deoxyxylo*-product **15**[2b] exclusively. The NMR spectra of the TBS-protected *deoxyribo*-derivative **16** and *deoxyxylo*-isomer **15** matched NMR data reported by Tronchet et al.[2b] (Scheme 4). This strategy was also successfully applied to deoxycytidine and deoxyadenosine systems to afford mixtures of *deoxyribo*- and *deoxyxylo*-isomers, in ∼4:1 ratios, which could also be isolated by chromatography. The products were derivatized to **17a**, **17b**, and **18** to minimize conformational equilibration[14]

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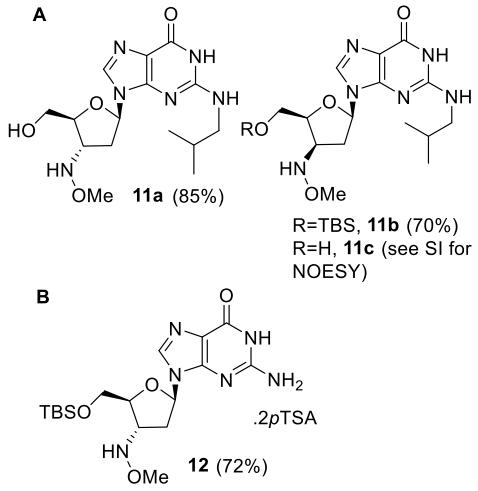


Figure 3. Deoxyguanosine systems. (A) The protecting groups of the isobutyroyl-protected imine substrates were also reduced. (B) DMT-protected imine substrate afforded the desired deoxyribo-configured methoxyamino-nucleoside upon DMT deprotection (*p*TSA = *para*-toluenesulfonate).

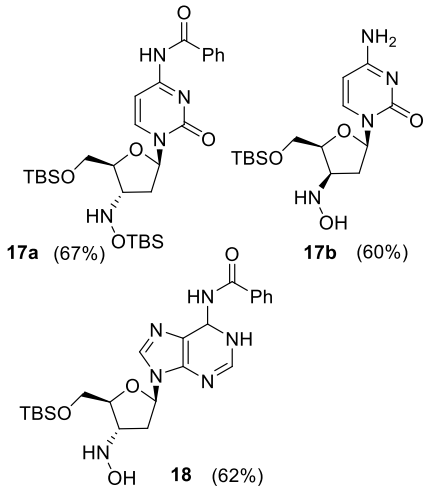


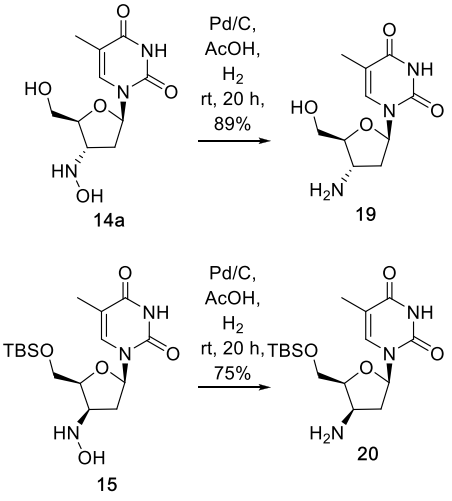
Figure 4. Product scope for deoxycytidine and deoxyadenosine systems.

and thus allow differentiation between the *deoxyribo*- and *deoxyxylo*-products through NOESY assignments. *Bis*-TBS-protected 3′-hydroxyamino-cytidine derivative **17a** exhibited NOESY correlations between the 3′-proton and the 6-(nucleobase)-proton, whereas the debenzoylated-*deoxyxylo*-derivative **17b** exhibited 1′-H to 3′-H NOESY correlation. Similarly, the TBS-protected-*deoxyribo*-3′-hydroxyamino-adenosine **18** exhibited NOESY correlations between the protons 3′- and 8-H of the nucleobase (Figure 4).

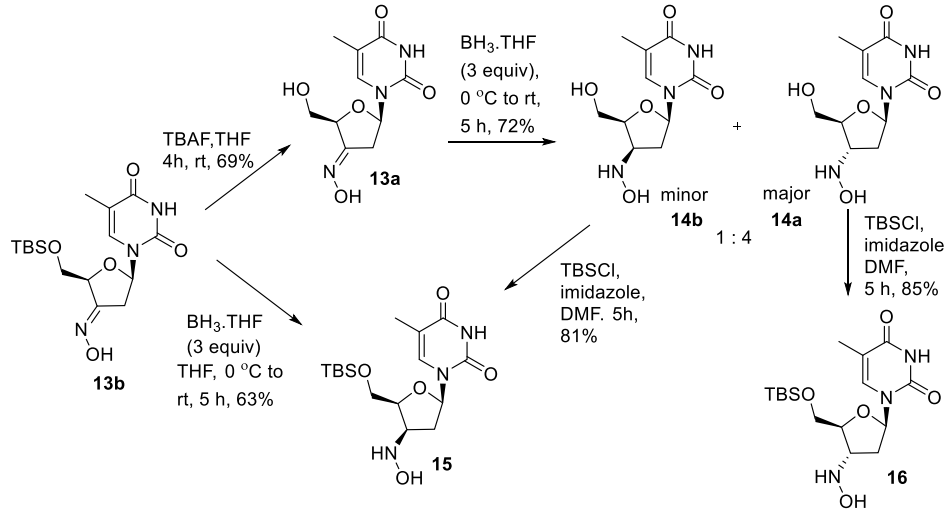
Kojima et al. demonstrated that 3′-hydroxylamine systems can be further reduced to 3′-amines by Pd/C and hydrogen to afford 3′-amino-ribonucleoside analogs.[16] We applied the same methodology to hydroxylamino-systems **14a** and **15**, and we were pleased to observe clean conversion to the corresponding amine systems **19** and **20** in 89% and 75% yield, respectively (Scheme 5).

In conclusion, we have developed efficient, direct strategies to obtain *deoxyribo*- and *deoxyxylo*-isomers of 3′-methoxyamino- and 3′-hydroxyamino-deoxynucleosides, from common

Scheme 5. Synthesis of 3′-Aminonucleoside Systems via Catalytic Reductions of Hydroxylamines



Scheme 4. Synthesis of *Deoxyribo*- and *Deoxyxylo*-Configured 3′-Hydroxyamino Thymidine Derivative



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intermediates, via stereoselective reductions of the corresponding 3′-imino deoxynucleosides using BH_3·THF. Our approach has delivered *ribo*-configured deoxynucleosides in good yields, which are otherwise difficult to obtain. To the best of our knowledge, the *ribo*-deoxycytidine derivative **9a**, deoxyadenosine derivative **10**, and *ribo*- and *xylo*-deoxyguanosine derivatives **11a−c** and **12** containing the 3′-methoxyamino-functionality are novel compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b03474.

Experimental procedures and characterizations (PDF)

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Notes

The authors declare no competing financial interest.

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